

Female plumage spottiness signals parasite resistance in the barn owl (*Tyto alba*)

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The hypothesis that extravagant ornaments signal parasite resistance has received support in several species for ornamented males but more rarely for ornamented females. However, recent theories have proposed that females should often be under sexual selection, and therefore females may signal the heritable capacity to resist parasites. We investigated this hypothesis in the socially monogamous barn owl, *Tyto alba*, in which females exhibit on average more and larger black spots on the plumage than males, and in which males were suggested to choose a mate with respect to female plumage spottiness. We hypothesized that the proportion of the plumage surface covered by black spots signals parasite resistance. In line with this hypothesis, we found that the ectoparasitic fly, *Carnus hemapterus*, was less abundant on young raised by more heavily spotted females and those flies were less fecund. In an experiment, where entire clutches were cross-fostered between nests, we found that the fecundity of the flies collected on nestlings was negatively correlated with the genetic mother's plumage spottiness. These results suggest that the ability to resist parasites covaries with the extent of female plumage spottiness. Among females collected dead along roads, those with a lot of black spots had a small bursa of Fabricius. Given that parasites trigger the development of this immune organ, this observation further suggests that more spotted females are usually less parasitized. The same analyses performed on male plumage spottiness all provided non-significant results. To our knowledge, this study is the first one showing that a heritable secondary sexual characteristics displayed by females reflects parasite resistance. **Key words:** bursa of Fabricius, *Carnus hemapterus*, fecundity, female plumage ornamentation, good gene, parasite resistance, *Tyto alba*. [*Behav Ecol* 12:103–110 (2001)]

Hamilton and Zuk (1982) proposed that individuals that are more resistant against pathogen attacks develop more extravagant ornaments. They assumed that the ability to resist the invasion of parasites is heritable and the expression of ornaments is condition-dependent, so that individuals that possess genes conferring lower susceptibility to parasites can invest more energy in the production of ornamental traits (Hamilton and Zuk, 1982; Zahavi, 1975). Thus, the cost to produce ornaments would allow individuals to honestly signal to potential mates current health state and/or intrinsic properties to resist parasites (Clayton, 1991; Kodrik-Brown and Brown, 1984; Zahavi, 1975). Later, Folstad and Karter (1992) proposed, with the “immunocompetence handicap hypothesis,” that the cost to producing an ornament is mediated by circulating sex-hormones. For instance, testosterone can induce the production of large male ornament and simultaneously depress the full immunological capacity to eliminate pathogens or to control the level of pathogen infection (Hillgarth and Wingfield, 1997; Salvador et al., 1996; but see Braude et al., 1999). Under this hypothesis the sex with a higher concentration of circulating sex-hormones should be more susceptible to parasites, and only high quality individuals may cope with the immunosuppressive effects of producing large ornaments (Saino et al., 1995).

Because males are usually under stronger sexual selection than females, and almost always more ornamented (Andersson, 1994), researchers focused mainly on males when study-

ing the relationship between morphological traits, parasite resistance and mate choice (e.g., Møller, 1990; Møller et al., 1998; Zuk, 1991). The best evidence so far for parasite-mediated sexual selection has been found in the barn swallow, *Hirundo rustica*. In this species, long-tailed males are preferred by females both to form a pair bond and to engage in extra-pair copulations (Møller, 1994). Møller (1990) also proposed that long-tailed males are preferred over short-tailed ones because they pass on to offspring good genes conferring resistance against parasites. At the interspecific level, Zuk (1991) found that species displaying a brighter plumage were more often infected by blood parasites suggesting that those bird species are at a higher selection pressure to develop bright plumage that signals parasite resistance.

Because males often transmit genes coding for an ornament into daughters, females may also exhibit the male trait but in a reduced state (Lande, 1980). Where a sex-trait can signal immunocompetence and improve mating success of males, it may not necessarily have a signal function when expressed in females (Cuervo et al., 1996; Hill, 1993; Muma and Weatherhead, 1989; Tella et al., 1997; but see Amundsen et al., 1997; Jones and Hunter, 1993, 1999; Møller, 1993; Potti and Merino, 1996). Even if females displaying a male trait have higher viability or achieve higher annual reproductive success than unadorned females (Møller, 1993), this does not necessarily imply that males exert sexual selection on them to express this trait (Cuervo et al., 1996). Indeed, the covariation between life-history traits and expression of a male ornament within females can be the mere consequence of a genetic correlation between the sexes for both properties (Halliday and Arnold, 1987; Lande, 1980), that is both sons and daughters may be simultaneously immunocompetent and ornamented but only sons will be chosen as a mate for those characteristics. Indeed, if the cost of mate choice is high for males, males

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may not have the possibility to exert strong sexual selection on females (Johnstone et al., 1996). Thus, the study of female signals of immunocompetence may be facilitated in species in which females display a morphological trait to a larger extent than males, because the larger expression of the trait may result from sexual selection exerted on females rather than on males.

In this context, the barn owl is a relevant model organism, because plumage varies from immaculate to heavily marked with black spots, and females usually display more and larger spots than males (Roulin, 1999a). This trait is heritable and its expression was not found to be mediated environmentally or to be condition-dependent (Roulin et al., 1998). Thus, plumage spottiness may potentially reflect the possession of good genes that improve the control of parasite infestation. And, because males that changed mates between two breeding attempts acquired a similarly spotted female as the previous one they were mated with, and the mates of sons and father resembled each other (Roulin, 1999b), the assessment of plumage spottiness may allow males to select females possessing good genes. In support of this proposition, the extent of female plumage spottiness was experimentally shown to reflect the offspring ability to mount an immune response towards an artificially administered antigen (Roulin et al., 2000).

The objectives of our study were to test the hypothesis that female plumage spottiness reflects parasite resistance. Using both observational and experimental approaches, we investigated in a Swiss and a French population the relationship between plumage spottiness and: (1) the offspring's burden of ectoparasitic flies *Carnus hemapterus*, (2) the fecundity of this ectoparasite, and (3) the size of an immune organ (bursa of Fabricius) of birds found dead along roads.

METHODS

The studied organisms and the bursa of Fabricius

Nests of barn owls are located in cavities, and composed of straw, dust, and pellets. Parents do not bring any material in their nest, but females frequently clean it by removing pellets. Young defecate in the nest and as a result, the odor is often ammoniac. Clutch size ranges from two to 11 eggs, brood reduction is frequent and nestlings spend 2 months in the nest before fledging (personal observation). Sex-roles in reproduction are well defined with the mother brooding the young and distributing small mammals brought by her partner (Baudvin, 1986). When the young are about 3 weeks old, the mother provides to the brood one-third of the food (Roulin, 1999b).

The ectoparasite *C. hemapterus* is a 2 mm long blood-sucking fly that parasitizes nestling birds (Dawson and Bortolotti, 1997; Kirkpatrick and Colvin, 1989; Roulin, 1998; Walter and Hudde, 1987). They emerge when the eggs of their hosts hatch and they lose their wings before reproducing. Parasite prevalence and intensity are high in the barn owl with 94% of the nestlings being infested with on average 40 flies per nestling (Roulin, 1998). Whereas flies are more abundant in nest boxes used the previous year by a family of barn owls than in boxes previously unused, their fecundity is unaffected by the status of nest boxes (Roulin, 1998, 1999c).

The bursa of Fabricius is an organ located just beside the cloaca. It grows during the nestling stage, and regresses prior to sexual maturity. In young birds (in chickens up to 2-weeks-old), the main production site of B cells is the bursa of Fabricius, and later B cells are produced not only by this organ but by the thymus and the bone marrow. Therefore, this organ is essential for immune defense in juveniles. The size of

the bursa of Fabricius reflects its cell population, and thus its antibody production. Interindividual differences in the size of this organ can have both a genetic and a phenotypic component. Parasites promote the development of the bursa of Fabricius (all information in Glick, 1983; Møller et al., 1996, 1998).

Plumage spottiness, ectoparasite intensity and clutch size of *C. hemapterus*

General method

The study was performed in Switzerland (46°49' N, 06°56' E) in an area covering 190 km² and located at an altitude ranging from 430 to 520 m. Since 1987, 110 nest boxes have been fastened to barns where breeding adults and their offspring have been captured. Breeding females were recognized by the presence of a brood patch. The sex of the nestlings was determined using the CHD-gene method (for details see Roulin et al., 1999).

Plumage spottiness was assessed on all nestlings and breeding adults from 1994 to 1999. Within a 60 × 40 mm frame, AR counted the number of spots on the breast, belly, flanks and underside of the wings. The diameter of three to 24 spots was measured with callipers to the nearest 0.1 mm. For each body part, the percentage of the 2400 mm² surface covered by spots was calculated using the formula $100 \times \pi \times \text{number of spots} \times (\text{mean spot diameter}/2)^2 / 2400$. Values found for the left and right flanks were averaged, and the same procedure was applied for the left and right wings. Then, values of the four body regions were averaged. This mean value was square-root transformed, called "plumage spottiness," and used in the statistical analyses. The method of assessing plumage spottiness has already been shown to be accurate (Roulin, 1999b; Roulin et al., 1998).

Observational design

In 1996, AR counted the number of *C. hemapterus* on each nestling when the oldest nestling of the brood was 25 days old. The whole body was screened with the exception of places where flies were not visible such as the openings of the ears. For each brood, the mean number of flies per nestling was defined as "parasite intensity." The method of assessing ectoparasite intensity is reliable (Roulin, 1998). Nest boxes were not cleaned during the whole study period allowing pupae to overwinter in the nest of their host.

In 1998, 1458 gravid female *C. hemapterus* were collected on 184 nestlings from 36 nests. Their white abdomen makes them easily recognizable. Each fly was put separately in a small Eppendorf tube (1.5 ml) at 37°C. After 24 h, all ectoparasites were dead, and A.R. counted the eggs laid in the tubes. Mean clutch size was not significantly correlated with altitude (Pearson correlation: $r = .10$, $p = .55$, $n = 36$) and thus, we did not control for this factor in the statistical analyses.

Experimental design

In 1999, 34 nests were matched in pairs with approximately the same hatching date ($r = .98$, $p < .001$, $n = 17$) and clutch size ($r = .77$, $p < .001$, $n = 17$). After 3 weeks of incubation, the entire clutch of a nest was transferred in the nest of its pair, and vice-versa. In this way, all nestlings were raised by foster parents. We cross-fostered clutches instead of hatchlings to ensure that *C. hemapterus* are in contact with nestlings of only one origin. For the same reason, we performed a full cross-fostering (i.e., entire clutches were swapped between nests) instead of a partial cross-fostering (i.e., half of the eggs of a clutch are swapped), because it cannot be excluded that in a nest flies move from one nestling to another. Two nests were abandoned before the eggs hatched (6%), and because

of technical problems (the car used to visit nest boxes broke down) one nest could not be visited. Sample size is therefore 31 experimental nests.

One week after cross-fostering incubating females were captured and randomly assigned to one of two experimental treatments. (1) Females with reduced plumage spottiness: for 15 females, the tip of the feathers was cut off with a scalpel to remove black spots. After the manipulation, plumage spottiness was significantly reduced by 4.7 times (paired t test, $t_{14} = -9.9$, $p < .001$); (2) Control: for 16 females, feathers were not manipulated (for six of these females feathers were mutilated with a scalpel without removing black spots). Females belonging to the two treatments did not differ in initial plumage spottiness (i.e., spottiness before treatment assignment) (Student t test, $t_{29} = 0.19$, $p = .86$), in laying date ($t_{29} = 0.03$, $p = .97$) and they laid a same-sized clutch ($t_{29} = 0.39$, $p = .70$).

As soon as the older nestling was 20 days of age, the nest was checked every 5 days up to fledging. At each visit and on each nestling, up to five gravid female *C. hemapterus* were captured. The mean number of visits with capture of flies was 4.8 per nest (range: 3–7). In total, 12 to 141 flies were collected per nest, and summed over the 31 nests 2087 flies were sampled. Each fly was put in a small Eppendorf tube, put at 37°C during 24 h, and AR counted the eggs laid in the tubes. For each nest box visit and for each nestling, the mean number of eggs laid by *C. hemapterus* was calculated. Then, values found for all siblings were averaged thereby providing a mean clutch size for that nest box visit. Finally, the mean clutch size found at all visits were averaged providing a mean clutch size for that nest box. This last value was used in the statistical analyses.

Measurement of the bursa of Fabricius

Between 8 December 1996 and 5 August 1998, dead barn owls were collected daily on 350 km of the French National roads in the regions Champagne and Lorraine. Cadavers were put in a freezer until dissection for sex determination, analysis of stomach content and measurement of the bursa of Fabricius (the spleen was unfortunately not measured). All measurements have been taken on 27 March 1997 and 22 September 1998. Body mass is the mass measured at the day of collection minus the mass of the stomach content. As an univariate measure of body size, we considered bill length because it reflects reliably the size of the skeleton (correlation between bill length and culmen, $r = .89$, $p < .001$, $n = 81$; maxilla, $r = .49$, $p < .001$, $n = 77$; femur, $r = .42$, $p = .002$, $n = 54$; tarsus, $r = .26$, $p = .018$, $n = 81$; data from the Natural History Museum of Basel). Fresh weight of the bursa of Fabricius was measured to the nearest 0.1 g, and its length and width to the nearest 0.1 mm. In the statistical analyses, we used the value “length \times width” as an index of the size of this organ because it varied to a larger extent than the fresh mass (coefficient of variation 1.68 versus 1.19). Note that the surface of the bursa of Fabricius was strongly correlated with its fresh mass ($r = .91$, $p < .001$, $n = 103$). The date when birds were collected was not significantly correlated with plumage spottiness (Spearman correlation; female, $r_s = -.19$, $p > .20$, $n = 47$; male, $r_s = -.19$, $p > .20$, $n = 58$). However, since in birds this organ regresses from fledging to the first-year of age (Glick, 1983), we statistically controlled for date of cadaver collection (30 June is defined as day 0) to assess the relationship between the size of the bursa of Fabricius and plumage spottiness. Adult birds have no bursa of Fabricius (Glick, 1983), and to ensure that we consider only birds in their first-year of life, we used only individuals in which this organ was found.

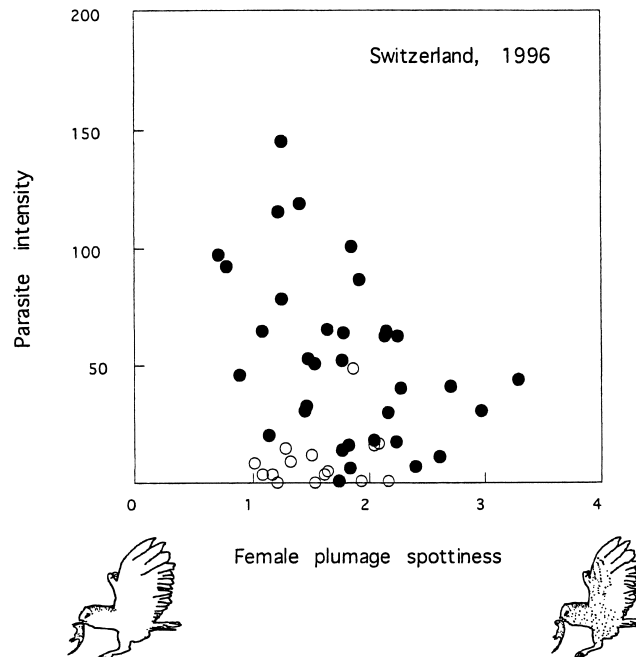


Figure 1
Relationship between the mean number of *C. hemapterus* per nestling and plumage spottiness of barn owl mothers. Filled and open circles show nest boxes occupied by a brood versus unoccupied in 1995.

Statistical procedure

Statistical analyses were performed using the Systat statistical package (Wilkinson, 1989). Because in some cases only the female parent was captured, sample sizes can differ between statistical analyses including mother and father plumage spottiness, and figures including only mother plumage spottiness. Statistical analyses were two-tailed.

RESULTS

Plumage spottiness and ectoparasite intensity

Nestlings were significantly more parasitized by *C. hemapterus* in nest boxes used than unused the previous year by a brood of barn owls and in nests where the mother was less heavily marked with black spots (ANCOVA: occupation of the nest box as a factor, $F_{1,44} = 25.3$, $p < .001$; mother plumage spottiness as a first covariate, $F_{1,44} = 4.8$, $p = .03$; father plumage spottiness as a second covariate, $F_{1,44} = 0.02$, $p = .68$) (Figure 1). Note that ectoparasite intensity was not significantly correlated with the date of its census ($r = -.23$, $p = .12$, $n = 50$), and brood size was not associated with female plumage spottiness ($r = -.01$, $p = .94$, $n = 50$). As can be seen in Figure 1, the relationship between parasite intensity and female plumage spottiness is evident only in nest boxes occupied by a family in 1995 ($r = -.44$, $p = .01$, $n = 34$; $r^2 = .19$) and not in nest boxes unoccupied the year before ($r = .24$, $p = .38$, $n = 16$). Because older females were spottier ($r = .43$, $p = .002$, $n = 50$), we controlled for female age in a partial correlation, and found that the negative relationship remained significant (female plumage spottiness, $r_{\text{partial}} = -.40$, $t_{31} = -2.46$, $p < .02$; female age, $r_{\text{partial}} = .04$, $t_{31} = 0.2$, $p > .50$).

One potential hypothesis accounting for this negative relationship is that more spotted females have access to nest boxes that are initially less infested by *C. hemapterus*. Two observa-

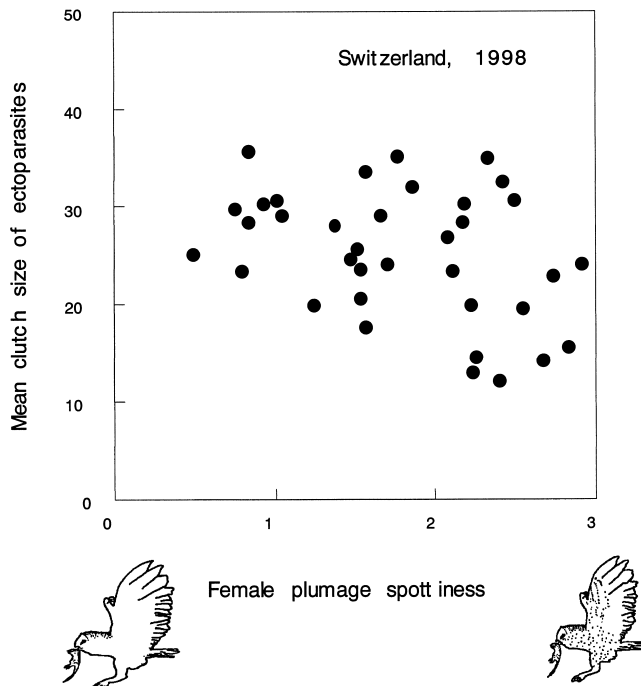


Figure 2
Relationship between the mean number of eggs laid by *C. hemapterus* and plumage spottiness of mothers in unmanipulated broods in 1998.

tions do not support this hypothesis. First, from 1994 to 1999 nest boxes not used the year before by a family of barn owls were not more often occupied by heavily than little spotted yearling females (Student *t* test, $t_{35} = 1.16$, $p = .25$). The age of these females was known with accuracy because they were ringed as nestlings. Second, by considering nest boxes in which the female breeding in 1997 was not the same as the one breeding in 1996, we found no significant correlation between ectoparasite intensities determined in nest boxes occupied in 1996 and plumage spottiness of the new 1997 female ($r = .11$, $p = .67$, $n = 17$).

Susceptibility to *C. hemapterus* did not appear to differ between the sexes, since female and male nest mates bore similar loads of flies (Wilcoxon matched-pairs signed-rank, $z = 0.09$, $p = .93$, $n = 36$) and brood sex-ratio was not correlated with ectoparasite intensity ($r = .07$, $p = .64$, $n = 50$). Although plumage spottiness of mothers reflected parasite intensity of their offspring, plumage spottiness of female offspring did not predict the intensity of parasitism. Indeed, more spotted female nestlings were not less parasitized than their less spotted female nest mates (comparison of mean ectoparasite loads of female nestlings above and below their median plumage spottiness, $z = 0.58$, $p = .56$, $n = 28$).

Plumage spottiness and clutch size of ectoparasites

Observations

In 1998, *C. hemapterus* laid clutches of smaller size when collected in the nest of more heavily spotted females (partial correlation between the mean number of eggs and mother plumage spottiness, $r_{\text{partial}} = -.37$, $t_{30} = -2.18$, $p < .05$ versus father plumage spottiness: $r_{\text{partial}} = .21$, $t_{30} = 0.84$, $p > .40$) (Figure 2). Because the production of eggs by *C. hemapterus* tended to increase with advancing date ($r = .32$, $p = .06$, $n = 36$), we calculated residuals from the regression of the mean number of eggs on date. The correlation between these

residuals and mother plumage spottiness was significantly negative ($r = -.34$, $p = .04$, $n = 36$). Note that in this sample female plumage spottiness was not correlated with female age ($r = .01$, $p = .95$, $n = 36$).

Fecundity of *C. hemapterus* was apparently not affected by the sex of their host, since *C. hemapterus* did not lay more eggs when collected on female nestlings than on their male nest mates (paired *t* test, $t_{22} = -0.52$, $p = .61$) and brood sex-ratio was not correlated with the fecundity of ectoparasites ($r = .02$, $p = .93$, $n = 36$). Although ectoparasitic fecundity was correlated with plumage spottiness of mothers, it was not with plumage spottiness of female nestlings. Flies collected on more spotted female nestlings were not less fecund than those collected on less spotted female nest mates (comparison between the mean number of eggs laid by flies collected on female nest mates above and below their median plumage spottiness, $z = 0.06$, $p = .95$, $n = 23$).

Experiment

Because in 1999 both fecundity of *C. hemapterus* and mother plumage spottiness were not correlated with the hatching date of the first barn owl's egg ($r = .16$, $p = .38$, $n = 31$ versus $r = .13$, $p = .48$, $n = 31$), we did not control for date in the statistical analyses. Note that plumage spottiness of foster and genetic fathers were not significantly correlated with the fecundity of parasites ($r = .25$, $p = .19$, $n = 30$ versus $r = -.19$, $p = .33$, $n = 28$), and that the mean clutch size of *C. hemapterus* captured at different days in the same nest was repeatable, but not to a large extent (repeatability, $24\% \pm 9\%$, $F_{30,113} = 2.57$, $p < .001$) as already found in 1998 (Roulin, 1999c). Thus, a larger number of nest box visits with collection of *C. hemapterus* may provide a more accurate approximation of ectoparasite fecundity. Because the number of visits was not significantly correlated with plumage spottiness of foster ($r = -.03$, $p = .88$, $n = 31$) and genetic mothers ($r = -.26$, $p = .16$, $n = 31$), and did not differ between the treatment "female with reduced plumage spottiness" and "control" (Mann-Whitney *U* test: $U = 110$, $p = .67$, $n = 15, 16$), variation in the number of nest box visits with capture of ectoparasites is unlikely to have biased the results.

In an ANCOVA model, ectoparasite fecundity was the dependent variable, the treatments "females with reduced plumage spottiness" and "control" as a factor, and plumage spottiness of foster and genetic mothers covariates. Treatment had no significant effect on ectoparasitic fecundity ($F_{1,27} = 0.36$, $p = .55$), initial plumage spottiness of foster mothers was not significantly related to the mean fecundity of *C. hemapterus* ($F_{1,27} = 1.07$, $p = .31$), and we found a significant effect of initial plumage spottiness of genetic mothers on the mean number of eggs the collected flies laid ($F_{1,27} = 4.41$, $p = .04$) (Figure 3).

Plumage spottiness and the size of the bursa of Fabricius

Male and female juveniles did not differ significantly in the size of their bursa of Fabricius (ANCOVA: sex factor, $F_{1,101} = 0.08$, $p = .78$), and this organ regressed from the first summer onwards (date as a covariate, $F_{1,101} = 56.7$, $p < .001$) in a similar magnitude in the two sexes (sex by date interaction, $F_{1,101} = 2.37$, $p = .13$) (Figure 4). For each sex, we calculated residuals from the regression of the size of this organ on the date when birds were collected. We found that more heavily spotted females had a smaller bursa of Fabricius ($r = -.47$, $p = .0008$, $n = 47$, $r^2 = .22$) (Figure 5). Same relationship did not hold in males ($r = .09$, $p = .51$, $n = 58$). We did not control for body size, because within each sex both residual size of the bursa of Fabricius and plumage spottiness were not

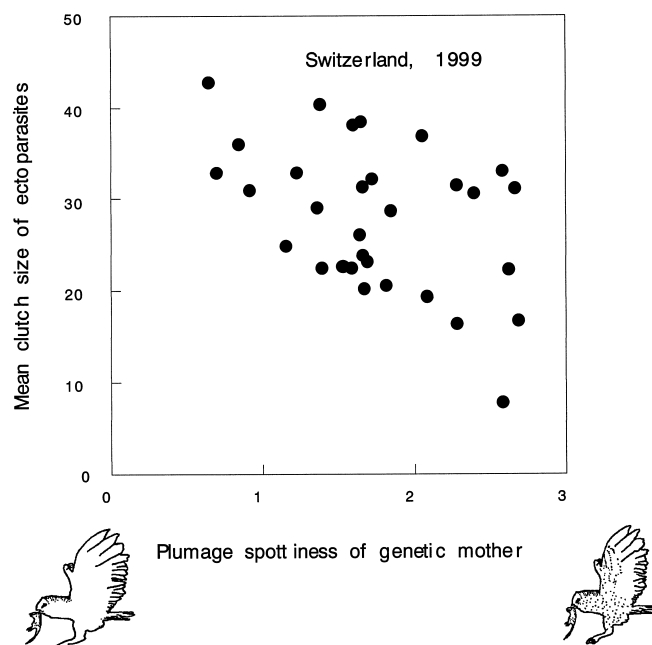


Figure 3
Relationship between the mean number of eggs laid by *C. hemapterus* collected on cross-fostered nestlings and plumage spottiness of genetic mothers in 1999. The Pearson correlation between the two variables is $r = -.45$, $p = .01$, $n = 31$ ($r^2 = .21$).

significantly correlated with body mass (Pearson correlation, p -values $> .32$) and bill length (p -values $> .29$).

DISCUSSION

Prior to our study, researchers have found that more ornamented male birds are less infested by ecto- (Møller, 1991) and endoparasites (Höglund et al., 1992; Wiehn et al., 1997), produce offspring that are less infested by ectoparasites (Møller, 1990), and have a smaller bursa of Fabricius (Møller et al., 1996) and spleen, another organ responsible for the production of lymphocytes (Møller et al., 1998). Thus, males that are more resistant against invasion of parasites are commonly more brightly feathered. An exception to this male-bias in signaling parasite resistance is the study of Potti and Merino (1996). They observed that female pied flycatchers, *Ficedula hypoleuca*, not infected by trypanosomes displayed a male secondary sexual character (forehead white patch) to a larger extent than infected females. Because in the barn owl females usually exhibit more and larger black spots than males, and males pair non-randomly with respect to female plumage spottiness (Roulin, 1999b), we investigated the hypothesis that female plumage spottiness reflects parasite resistance. Both observational and experimental results supported this hypothesis. In Switzerland, we found that ectoparasite intensity and fecundity of ectoparasites were lower when the mother was more heavily spotted. A cross-fostering experiment demonstrated that offspring of more heavily spotted females have the pre-determined ability to impair the fecundity of ectoparasites. Finally, in France more heavily spotted females had a smaller bursa of Fabricius.

Ectoparasite intensity and female plumage spottiness

At least four potential mechanisms can explain the fact that offspring of more spotted mothers supported a lower burden of ectoparasites. First, females may be not randomly distrib-

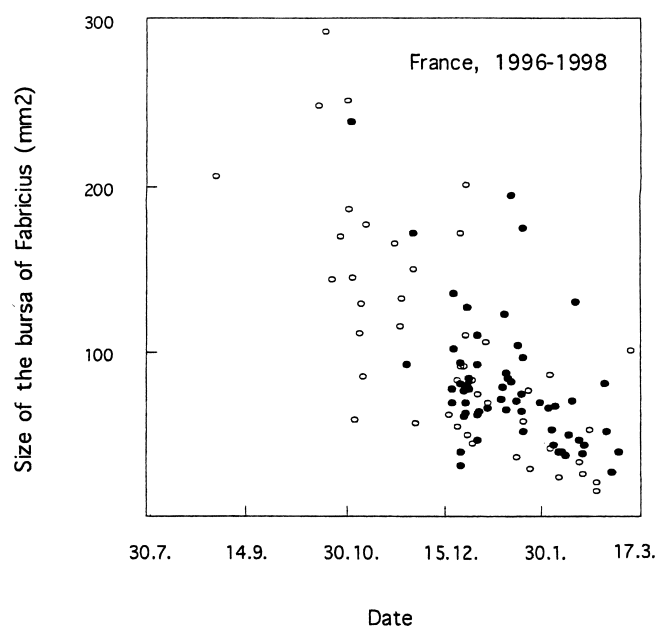


Figure 4
Size of the bursa of Fabricius (length \times width) in relation to the date when birds were collected dead. Filled circles represent males and open circles females.

uted among environments with more spotted females occupying nest boxes containing a naturally lower number of ectoparasites. The observation showing that nest boxes containing a small number of parasites the year before were not preferentially used by more heavily spotted females suggests that this explanation does not hold.

Second, at night females often clean the nest by removing detritus within which *C. hemapterus* are often located (Roulin

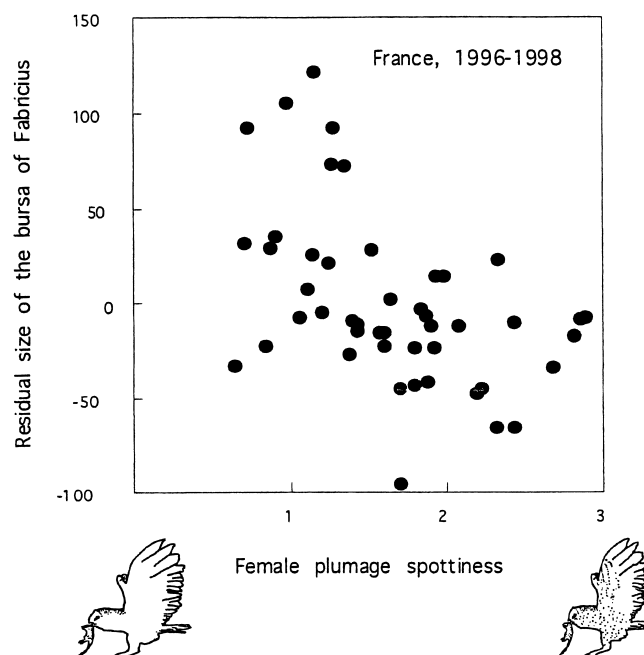


Figure 5
Relationship between the residual size of the bursa of Fabricius (i.e., the size of this organ corrected for the date when individuals were collected dead) and female plumage spottiness.

A, personal observation). Therefore, results may be explained by a more intense nest sanitation by more spotted females. Because the time invested in nest sanitation may be traded against other activities (Christe et al., 1996) such as feeding rate, this second hypothesis would predict that offspring of more spotted females are fed at a lower rate. However, another study indicated that offspring of more spotted females were fed at higher rates (Roulin, 1999b). Thus, the nest sanitation hypothesis may not be a likely one unless more spotted females are better individuals at all breeding related activities.

Third, because nestlings raised by little spotted females were in worse condition than those raised by heavily spotted females (Roulin, 1999b), we may have detected a negative relationship between ectoparasite intensity and female plumage spottiness if parasites build up a larger population when their hosts are in poorer condition. However, given that brood size manipulations significantly affected nestling body condition (Roulin et al., 1999) without altering ectoparasite intensity (Roulin, 1998), this third explanation can be rejected.

Finally, a previous study showed that plumage spottiness is heritable (Roulin et al., 1998), and following sexual selection theory secondary sexual characteristics may have evolved to signal the heritable ability to resist parasites (Hamilton and Zuk, 1982). Thus, ectoparasites were less numerous in broods of heavily spotted females perhaps because offspring have the pre-determined ability to resist invasion of ectoparasites so that *C. hemapterus* may have more difficulties to build up a large population. Since the quantity of winged flies remains constant throughout the rearing period and since the total number of flies met in a brood is larger in experimentally enlarged than reduced broods (Roulin, 1998), several generations of flies may be produced during a single barn owl breeding attempt. In this context, we predicted that the fecundity of ectoparasites to be lower when feeding on the offspring of heavily spotted mothers.

Fecundity of *C. hemapterus* and female plumage spottiness

To our knowledge the present study is the first one to measure the fecundity of parasites in relation to secondary sexual characteristics. The idea behind this approach is that reproductive success of parasites is likely to depend more strongly on host characteristics than parasite intensity. Indeed, while fecundity may be related to the intake rate of high quality food extracted by parasites from their host (Brossard and Girardin, 1979; Brossard et al., 1991), population size of ectoparasites depends also on other factors including immigration and previous occupation status of nest boxes (Roulin, 1998). In agreement with the good gene theory of sexual selection (Hamilton and Zuk, 1982), we found that *C. hemapterus* collected on cross-fostered nestlings were less fecund when the genetic mother was more heavily spotted. At least three mechanisms may cause such a reduction of the fecundity.

The first mechanism relies on the fact that if males invest more effort in reproduction when mated to more heavily spotted females (Roulin, 1999), such pairs would produce offspring in higher condition that can also invest more energy to combat parasites. This hypothesis would suggest that the relationship between the fecundity of ectoparasites and female plumage spottiness has a phenotypic rather than a genotypic basis. This is unlikely because nestling phenotypic condition did not affect the fecundity of *C. hemapterus*, as suggested by the absence of significant correlations between ectoparasitic egg output and several indexes of nestling condition (Roulin, 1999c). Furthermore, since plumage spottiness of foster mothers was not correlated with the fecundity of ectoparasites, more spotted females probably did not occupy territories where parasites are characterized by a low fe-

cundity, and those mothers did not provide high quality parental care that could have reduced the fecundity of *C. hemapterus*. Finally, the experimental manipulation of female plumage spottiness did not affect parasites.

The second mechanism is based on the knowledge that biting insects, including mosquitos and ticks are limited by the skin thickness/composition of their host to extract large amounts of blood (Lehane, 1991). *C. hemapterus* feed on the blood of their host (Kirkpatrick and Colvin, 1989), and hence one may speculate that young of more spotted females possess, for instance, a thicker skin preventing flies from efficiently taking blood meals. This hypothesis has not yet been investigated.

The third mechanism proposes that more spotted females have a more efficient immunological system. It has been shown that ectoparasites elicit an immune response of their host (Allen and Nelson, 1982). For instance, ticks fed on the blood of immunologically resistant rabbits obtained smaller bloodmeals and produced fewer eggs (Brossard and Girardin, 1979). Moreover, in the barn swallow more ornamented males mounted higher immunological response towards injection of sheep red blood cells (Saino and Møller, 1996) suggesting that plumage ornamentation may signal the ability to mount higher immunological defences to resist parasites. In this context, we measured the size of the bursa of Fabricius to get an index of the immune status of barn owls in relation to plumage spottiness. We predicted that more spotted females should develop a smaller bursa of Fabricius by analogy to the study of Møller et al. (1996) showing that more ornamented male house sparrows, *Passer domesticus*, were less infested by a mallophagan and had a smaller bursa of Fabricius. Our hypothesis was supported. Aware that other interpretations of this result are potentially plausible, we performed a more stringent test of this hypothesis (Roulin et al., 2000). The idea of this test was that although offspring of more spotted females are less parasitized, and hence less challenged immunologically, once challenged they would be more efficient at clearing pathogens. Using artificial injection of sheep red blood cells, cross-fostered offspring of more heavily spotted mothers were more capable to mount an humoral immune response (Roulin et al., 2000).

Implication for the good gene theory of sexual selection

The present study and a previous one (Roulin et al., 2000) suggest that female plumage spottiness signals the heritable capacity to resist parasites and pathogens. Resistance may be inherited via genes (i.e., heavily spotted mothers transmit genes into offspring and when active they confer resistance) or via substances included in the eggs that improve offspring resistance (maternal effect). These two possibilities are not mutually exclusive, and results in the barn owl may suggest that the resistance conferred to offspring is a maternal effect that depends on whether mothers have good genes or not. This speculation arises from two observations. First, since: (1) *C. hemapterus* does not feed on adults, (2) *C. hemapterus* emerge when barn owl nestlings hatch (Kirkpatrick and Colvin, 1989; Roulin, 1998), and (3) more heavily spotted females do not occupy nests where the initial number of flies is higher, more spotted females are probably not better vaccinated against *C. hemapterus*. Therefore, if more heavily spotted mothers include in their eggs more molecules (e.g., antibodies) specifically directed against *C. hemapterus*, this may not be because such females had been vaccinated against *C. hemapterus*, but probably because they have good genes. Given that more spotted mothers also conferred to offspring immune protection against an artificially administrated antigen (Roulin et al., 2000), these good genes may improve the over-

all pathogen/parasite resistance of offspring. Second, if the pathogen/parasite resistance resulted from the expression of good genes in offspring, we should have expected that (1) compared to male nestlings, females nestlings are parasitized by a lower number of *C. hemapterus* that have also a lower fecundity, and they mount a more intense immune response against an antigen, and (2) more spotted female nestlings are parasitized by a lower number of less fecund flies than their less spotted female siblings, and they mount a more intense immune response against an antigen. These two predictions were not supported (Roulin et al., 2000; present study) suggesting that females may transfer in their eggs good gene products that confer better overall immune protection to their offspring. This speculation should motivate detailed research on the mechanisms of the good gene theory of sexual selection.

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REFERENCES

- Allen JR, Nelson WA, 1982. Immunological responses to ectoparasites. *Fortschritte der Zoologie* 27:169–180.
- Amundsen T, Forsgren E, Hansen LTT, 1997. On the function of female ornaments: male bluethroats prefer colourful females. *Proc R Soc Lond B* 264:1579–1586.
- Andersson M, 1994. Sexual selection. Princeton, New Jersey: Princeton University Press.
- Baudvin H, 1986. La reproduction chez la chouette effraie (*Tyto alba*). *Le Jean le Blanc* 25:1–25.
- Braude S, Tang-Martinez Z, Taylor GT, 1999. Stress, testosterone, and the immunoredistribution hypothesis. *Behav Ecol* 10:345–350.
- Brossard M, Girardin P, 1979. Passive transfer of resistance in rabbits infested with adult *Ixodes ricinus* L.: humoral factors influence feeding and egg laying. *Experientia* 35:1395–1397.
- Brossard M, Rutti B, Haug T, 1991. Immunological relationships between host and ixodid ticks. In: *Parasite-host associations. Coexistence or conflict?* (Toft CA, Aeschlimann A, eds). Oxford University Press, Oxford; 177–200.
- Christe P, Richner H, Oppliger A, 1996. Of great tits and fleas: sleep baby sleep. . . *Anim Behav* 52:1087–1092.
- Clayton DH, 1991. The influence of parasites on host sexual selection. *Parasitol Today* 7:329–334.
- Cuervo JJ, Lope de F, Möller AP, 1996. The function of long tails in female barn swallows (*Hirundo rustica*): an experimental study. *Behav Ecol* 7:132–136.
- Dawson RD, Bortolotti GR, 1997. Ecology of parasitism of nestling American kestrels by *Carnus hemapterus* (Diptera, Carnidae). *Can J Zool* 75:109–112.
- Folstad I, Karter AJ, 1992. Parasites, bright males, and the immunocompetence handicap. *Am Nat* 139:603–622.
- Glick B, 1983. Bursa of Fabricius. In: *Avian biology Vol 7* (Farner DS, King JR, Parkes KC, eds). New York: Academic Press; 443–500.
- Halliday T, Arnold SJ, 1987. Multiple mating by females: a perspective from quantitative genetics. *Anim Behav* 35:939–941.
- Hamilton WD, Zuk M, 1982. Heritable true fitness and bright birds: a role for parasites? *Science* 218:384–387.
- Hill GE, 1993. Male mate choice and the evolution of female plumage coloration in the house finch. *Evolution* 47:1515–1525.
- Hillgarth N, Wingfield JC, 1997. Parasite-mediated sexual selection: endocrine aspects. In: *Host-parasite evolution. General principle and avian models* (Clayton DH, Moore J, eds). Oxford: Oxford University Press; 78–104.
- Höglund J, Alatalo RV, Lundberg A, 1992. The effects of parasites on male ornaments and female choice in the lek-breeding black grouse (*Tetrao tetrix*). *Behav Ecol Sociobiol* 30:71–76.
- Johnstone RA, Reynolds JD, Deutsch JC, 1996. Mutual mate choice and sex differences in choosiness. *Evolution* 50:1382–1391.
- Jones IL, Hunter FM, 1993. Mutual sexual selection in a monogamous seabird. *Nature* 362:238–239.
- Jones IL, Hunter FM, 1999. Experimental evidence for mutual inter- and intrasexual selection favouring a crested auklet ornament. *Anim Behav* 57:521–529.
- Kirkpatrick CE, Colvin BA, 1989. Ectoparasitic fly *Carnus hemapterus* (Diptera: Carnidae) in a nesting population of common barn-owls (Strigiformes: Tytonidae). *J Med Entomol* 26:109–112.
- Kodric-Brown A, Brown JH, 1984. Truth in advertising: the kinds of traits favored by sexual selection. *Am Nat* 124:309–323.
- Lande R, 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* 34:292–305.
- Lehane MJ, 1991. Biology of blood-sucking insects. London: Harper Collins Academic.
- Møller Ap, 1990. Effects of a haematophagous mite on the barn swallow (*Hirundo rustica*): a test of the Hamilton and Zuk hypothesis. *Evolution* 44:771–784.
- Møller Ap, 1991. Parasites, sexual ornaments, and mate choice in the barn swallow. In: *Bird-parasite interactions: ecology, evolution, and behaviour* (Loye JE, Zuk M, eds). Oxford: Oxford University Press; 328–343.
- Møller Ap, 1993. Sexual selection in the barn swallow *Hirundo rustica*, Vol. III. Female tail ornaments. *Evolution* 47:417–431.
- Møller Ap, 1994. Sexual selection and the barn swallow. Oxford: Oxford University Press.
- Møller Ap, Dufva R, Erritzøe J, 1998. Host immune function and sexual selection in birds. *J Evol Biol* 11:703–719.
- Møller Ap, Kimball RT, Erritzøe J, 1996. Sexual ornamentation, condition, and immune defence in the house sparrow *Passer domesticus*. *Behav Ecol Sociobiol* 39:317–322.
- Muma KE, Weatherhead PJ, 1989. Male traits expressed in females: direct or indirect sexual selection? *Behav Ecol Sociobiol* 25:23–31.
- Potti J, Merino S, 1996. Decreased levels of blood trypanosome infection correlate with female expression of a male secondary sexual trait: implications for sexual selection. *Proc R Soc Lond B* 263: 1199–1204.
- Roulin A, 1998. Cycle de reproduction et abondance du diptère parasite *Carnus hemapterus* dans les nichées de chouettes effraies *Tyto alba*. *Alauda* 66:265–272.
- Roulin A, 1999a. Delayed maturation of plumage coloration and plumage spottiness in the barn owl *Tyto alba*. *J Ornithol* 140:193–197.
- Roulin A, 1999b. Nonrandom pairing by male barn owls *Tyto alba* with respect to a female plumage trait. *Behav Ecol* 10:688–695.
- Roulin A, 1999c. Fécondité de la mouche *Carnus hemapterus*, parasite des jeunes chouettes effraies (*Tyto alba*). *Alauda* 67:205–212.
- Roulin A, Richner H, Ducrest A-L, 1998. Genetic, environmental and condition-dependent effects on female and male ornamentation in the barn owl *Tyto alba*. *Evolution* 52:1451–1460.
- Roulin A, Ducrest A-L, Dijkstra C, 1999. Effect of brood size manipulations on parents and offspring in the barn owl, *Tyto alba*. *Ardea* 87:91–100.
- Roulin A, Jungi TW, Pfister H, Dijkstra C, 2000. Female barn owls (*Tyto alba*) advertise good genes. *Proc R Soc Lond B* 267:937–941.
- Saino M, Möller AP, Bolzern AM, 1995. Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. *Behav Ecol* 6:397–404.
- Saino M, Möller A. P., 1996. Sexual ornamentation and immunocompetence in the barn swallow. *Behav Ecol* 7:227–232.
- Salvador A, Veiga JP, Martin J, Lopez P, Abellenda M, Puerta M, 1996. The cost of producing a sexual signal: testosterone increases the susceptibility of male lizards to ectoparasitic infestation. *Behav Ecol* 7:145–150.
- Tella JL, Forero MG, Donazar JA, Hiraldo F, 1997. Is the expression

- of male traits in female lesser kestrels related to sexual selection? *Ethology* 103:72–81.
- Walter G, Hudde H, 1987. Die Gefiederfliege *Carnus hemapterus* (Milichiidae, Diptera), ein Ektoparasit der Nestlinge. *J Ornithol* 128: 251–255.
- Wiehn J, Korpimäki E, Bildstein KL, Sorjonen J, 1997. Mate choice and reproductive success in the American kestrel: a role for blood parasites? *Ethology* 103:304–317.
- Wilkinson L, 1989. SYSTAT: the system for statistics. Evanston, Illinois: SYSTAT.
- Zahavi A, 1975. Mate selection—a selection for a handicap. *J Theor Biol* 53:205–214.
- Zuk M, 1991. Parasites and bright birds: new data and a new prediction. In: Bird-parasite interactions: ecology, evolution, and behaviour (Loye JE, Zuk M, eds). Oxford: Oxford University Press; 317–327.